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Oleaginous extract from the fruits *Pterodon pubescens* Benth induces antinociception in animal models of acute and chronic pain

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ABSTRACT

Ethnopharmacological relevance: *Pterodon pubescens* Benth is a medicinal plant commonly used for therapeutic purposes in folk medicine for rheumatic diseases' treatment. In the present work we analyzed the chemical composition of the oleaginous extract of *P. pubescens* Benth (OEPp) and extended the antinociceptive effect of OEPp evaluating its role on animal models of acute and chronic pain.
Materials and methods: The antinociceptive and antiedematogenic effects of OEPp (3–100 mg/kg, i.g.) were evaluated in the formalin test; mechanical allodynia in the postoperative pain and complex regional pain syndrome type-I (CRPS-I) animal models; and thermal hyperalgesia was induced by plantar incision. Finally, we performed a phytochemical analysis of OEPp.
Results: The chemical composition of OEPp was analyzed by mass spectrometry (GC/MS) and eight sesquiterpene compounds were identified, i.e. three major sesquiterpene (E-cariofilene, γ-muurolene, biciclogermacrene), and nine vouacapane diterpenes, four of which showed in major concentration (6α-acetoxyvouacapane, 6α,7β-dimetoxivouacapan-17-ene, 6α-acetoxy,7β-hidroxyvouacapane, 6α,7β-diacetoxycouacapane). Furthermore, the results of the present study demonstrate, for the first time, that the OEPp reduced mechanical allodynia in the postoperative pain and CRPS-I animal models. OEPp also increased the paw withdrawal latency in hot- and cold-plate tests in the postoperative pain model. In addition, the present work confirms and extends previous data from literature showing that systemic administration of OEPp caused significant inhibition against both phases of pain response to formalin intraplantar injection and edema formation.
Conclusions: Together, present and previous findings show that OEPp given intra-gastrically caused significant inhibition against both phases of formalin intraplantar injection and effectively inhibited mechanical and thermal hyperalgesia in the postoperative pain and CRPS-I animal models.

1. Introduction

It is estimated that the economic burden of treating chronic pain that develops from acute pain in a 30 year-old individual, over a life time, could be as much as $1 million (Cousins et al., 2000). The prevention and effective relief of acute pain may improve clinical outcomes, save health care resources and improve quality of life. Surgical incision in postoperative patients leads to mechanical hyperalgesia, therefore, mechanical evoked pain after surgery is important for postoperative pain, and the underlying mechanisms must be studied to improve postoperative analgesia and the outcome of patients after surgery (Pogatzki and Raja, 2003).

Chronic post-ischemic pain (CPIP) is an animal model of complex regional pain syndrome type I (CRPS-I). Clinically, CRPS can begin after an injury, usually in the extremities. This trauma may be a distal fracture, nerve lesion, post-surgical injury and others. The syndrome is divided into two types: with major nerve injury (type II) or without (type I) (Feliu and Edwards, 2010). Studies in rats show that hind paw ischemia and reperfusion (IR) causes microvascular injury (Coderre and Bennett, 2010) and symptoms that are similar to CRPS-I in humans. These findings reinforce the evidence that CRPS-I may depend, in part, on tissue ischemia (Coderre et al., 2004) in patients with microvascular pathologies that lead to chronic tissue inflammation (Laferrière et al., 2008).

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Medicinal plants are largely used worldwide by the population and have proved to be a rich source of new active compounds, especially to treat pain and inflammatory processes (Calixto et al., 2004). The medicinal plant *Pterodon pubescens* Benth is popularly known as "sucupira-branca" or "faveira" because its fruits form small "honeycombs" where its essential oil is stored. It is a native tree specie widely distributed throughout central Brazil (Lorenzi, 1998). This vegetal specie is used for therapeutic purposes, in folk medicine, for rheumatic diseases' treatment (Carvalho, 2004). It has been demonstrated that the hydroalcoholic extract of *P. pubescens* seeds (HEPp), administered orally, effectively inhibits edema and pain caused by collagen-induced arthritis (Sabino et al. 1999). Furthermore, Pinto Coelho et al. (2001) have demonstrated that the oral administration of HEPp reduced the arthritic index when compared to control group and concluded that HEPp presents antiarthritic activity, with no toxic effect in mice. Here, we examined: (i) the chemical composition of OEPp; (ii) the possible antihyperalgesic effect of OEPp in standard rodent models of acute and chronic pain, i.e., the intraplantar injection of formalin, postoperative pain-induced by plantar incision surgery (PIS), and chronic post-ischemic pain-induced by prolonged hind paw ischemia and reperfusion.

2. Material and Methods

2.1. Animals

All animal care and experimental procedures were carried out in accordance with the National Institutes of Health Animal Care Guidelines (NIH publications no. 80–23), and were approved by the Ethics Committee of the Universidade do Sul de Santa Catarina (protocol number 11.008.4.06.IV). Experiments were conducted using Swiss male mice (25–35 g), obtained from the Biotério Central da Universidade do Sul de Santa Catarina. Animals were housed at 22 ± 2 °C under a 12 hours light/12 hours dark cycle (lights on at 06:00) and with free access to food and water. Animals were acclimatized to the laboratory for at least 1 hour before testing and were used only once throughout the experiments. The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of drug treatments.

2.2. Extraction of vegetal material

The fruits of *P. pubescens* Benth were acquired in the medicinal garden of the Florianopolis' Health Pastoral, Santa Catarina, Brazil and identified by Dr. Rafael Trevisan (Department of Botany, Federal University of Santa Catarina, Florianópolis, Brazil). The dried fruits (with seed) of *P. pubescens* (100 g) were powdered and submitted to maceration with alcohol cereals (1 L) at room temperature. After 30 day, the extract was filtered and the solvent was evaporated under reduced pressure in a rotative evaporator to yield a oleaginous extract of *P. pubescens* (OEPp) (20,716 g) as previously described (Coelho et al., 2005).

2.3. Phytochemical analysis of the extract from P. pubescens

The oil was analyzed by chromatography-mass spectrometry (GC–MS). The analysis was performed in a GC–MS QP5050 Shimazdu (70 eV) apparatus, using DB-5 column (30 m × 0.25 mm × 0.25 μm) and helio carrier gas. The column temperature was programmed 60–280 °C (3 °C min) with isotherm 290 °C to 5/ min and flux of 1 mL/min. The data spectral were compared to the literature. The separation of major compounds was by flash chromatography column eluted with 20% hexane:acetate. And isolated compounds were subjected to spectrometry of ^{1}H, ^{13}C NMR and GC/MS analyses.

2.4. Formalin test

Formalin-induced nociception was measured as previously described (Hunskaar et al., 1985; Martins et al., 2011). Animals were injected with 20 μL of a 2.5% formalin solution (0.92% formaldehyde in saline) intraplantarly (i.pl.) in the ventral surface of the right hindpaw. Animals were observed for 30 min; the time between 0 and 5 min represented the neurogenic phase and the time between 15 and 30 min represented the inflammatory phase. Time spent licking the injected paw was recorded and was indicative of nociception. To determine the effects of OEPp administration on nociception, animals were treated with OEPp (1–100 mg/kg, i.g.) 1 hour before formalin injection in the right hindpaw. Control animals were treated with vehicle (5% tween 80 in saline, 10 mL/kg, i.g.) 1 hour before formalin injection. After the formalin injection, animals were immediately placed in individual glass cylinders (20 cm diameter). Antinociception was expressed as a reduction of time that treated group spent licking the paw in relationship to the control group.

The antiedematogenic effect of EOPp was assessed by measuring the external diameter of the hind paw using a vernier caliper (Starrett: 125MEB-6/150) after the assessment of licking caused by formalin. Measurements were obtained from each group of mice, and the edema difference for each animal was generated by subtracting the diameter of the left hind paw (saline injection) from the right hind paw (formalin solution).

2.5. Plantar incision surgery

The plantar incision surgery (PIS) was performed as previously described (Pogatzki and Raja, 2003). Briefly, mice were anesthetized with 1%–2% isoflurane delivered via a nose cone. After sterile preparation of the right hind paw, a 5 mm longitudinal incision was made through skin and fascia of the plantar surface using a number 11 scalpel blade. The incision started 2 mm from the proximal edge of the heel and extended toward the toes. The underlying muscle was elevated with curved forceps, leaving the muscle origin and insertion intact. After wound homeostasis, the skin was apposed with a 6.0 mm nylon mattress suture, and the wound was covered with 10% povidone–iodine solution. Control animals were anesthetized but no incision was made. Animals were allowed to recover in their cages, and sutures were removed on the second postoperative day.

2.6. Chronic post-ischemic pain

Chronic post-ischemic pain (CPIP) is an animal model of CRPS-I developed using a 3 hours ischemia-reperfusion injury of the rodent right hind paw. CPIP induction was performed as described previously (Millecamps et al., 2010). CPIP mice were generated following exposure to prolonged hind paw ischemia and reperfusion (IR). Mice were anesthetized over a 3 hours period with a bolus (7%, 0.6 mL/kg, i.p.) of chloral hydrate and 20% of the initial volume at the end of the first and second hour. After induction of anesthesia, an elastic O-ring for braces (Elástico Ligadura 000–1237, Uniden) with 1.2 mm internal diameter was placed around the mouse's right hind limb just proximal to the ankle joint. The O-rings were selected to provide a tight-fit that produced ischemia. They were left on the limb for 3 hours as initially described with larger O-rings. The O-ring was always positioned at a point on the limb just proximal to the medial malleolus and its application was standardized by sliding it off the outside of a 100 μL pipette tip (that had 4 mm of the

larger end cut) after the hind paw was inserted into the pipette as far as possible. Sham mice received exactly the same treatment except that the O-ring was cut so that it only loosely surrounded the ankle and did not occlude blood flow to the right hind paw.

2.7. Mechanical allodynia

The mechanical allodynia was measured as described previously (Bortalanza et al., 2002). Mice were acclimated in individual clear boxes ($9 \times 7 \times 11\ cm^3$) on an elevated wire mesh (6 mm) platform (70×40 cm) to allow access to the ventral surface of the hind paws. The right hind paw was stimulated with a constant pressure of 0.4 g von Frey filaments (VFF) (Stoelting, Chicago, USA). The response frequency to 10 applications was taken as the nociceptive behavior. The results are expressed as the percentage of withdrawal response. The day before surgery, the animals were subjected for testing to characterize the baseline response. Only animals that showed a response rate around 20% were selected.

In postoperative pain (24 hours after surgery) or CPIP (2nd and 7th days after surgery) models we investigated the time course of OEPp (0.1–100 mg/kg, i.g.) antinociceptive effect, the animals were evaluated 1, 2 and 4 hours after treatment. Control animals received a similar volume of vehicle (10 mL/kg, i.g.). To investigate the effects of long-term treatment with OEPp (100 mg/kg, i.g.), the treatment was repeated for 6 consecutive days (days 1st–6th) after PIS.

In the model of CPIP, to investigate the effects of long-term treatment OEPs (100 mg/kg) or vehicle were administered intragastric to mice once a day for 5 consecutive days (from 7th to 11th day after IR). The treatment was interrupted and reinitiated on 14th day after the IR and continued until the 15th day. Mechanical allodynia was measured every day, always 1 hour after OEPp (100 mg/kg, i.g.) treatment (postoperative pain and CPIP models).

2.8. Thermal hyperalgesia

To assess thermal hyperalgesia to cold and hot stimulus in mice, the Cold/Hot Plate Analgesia Meter (Columbus Instruments, USA) was used according to a minor modification of the method described by Bennett and Xie (1988). Mice were placed in clear plastic chambers ($7 \times 9 \times 11\ cm^3$) on an elevated surface. They were acclimatized to the environment for 1 hour before testing. To analyze cold thermal hyperalgesia mice were placed on the cold plate ($10 \pm 1\ ^\circ C$) and to analyze heat thermal hyperalgesia mice were placed in the hot plate ($48 \pm 1\ ^\circ C$). Mice were treated with OEPp (100 mg/kg, i.g.) or vehicle (10 mL/kg, i.g.) 1 hour beforehand. The nociception was evaluated by the right hindpaw withdrawal latency. The cut-off latency for cold plate test was 120 seconds and for hot plate was 60 seconds. The nociceptive behavior was tested 24 hours after PIS.

2.9. Measurement of locomotor activity

To evaluate the effect of OEPp on spontaneous locomotor activity, mice were submitted to the open field test (Martins et al., 2011). The open-field apparatus consisted of a wooden box measuring $40 \times 60 \times 50$ cm. The floor of the arena was divided into 12 equal squares, and the number of squares crossed by the animal with all paws was counted in a 6 min session. Mice were treated with OEPp (1–100 mg/kg, i.g.) or vehicle (10 mL/kg, i.g.) 1 hour beforehand.

2.10. Statistical analysis

Data are presented as means ± standard error of the mean (S.E.M.). The ID_{50} value was determined from experiment using nonlinear regression GraphPad software (GraphPad Software, Inc., San Diego, CA). Formalin and open field test was performed by 1-way ANOVA followed by the Newman–Keuls' test, when appropriate CPIP and postoperative pain testing was compared using two-way analysis of variance (ANOVA) for repeated measures, with Bonferroni's multiple comparison as the post-hoc test. *P*-values that were less than .05 were considered significant.

3. Results

3.1. Phytochemical analysis of the extract from P. pubescens

The chemical analysis of the OEPp sample used in the present investigations showed two distinct regions, in the sesquiterpene (RT between 25 and 33 min) and diterpene (RT between 55 and 70 min) regions, representing seventeen compounds of the total oil content (Fig. 1A). This work focused in a special kind of diterpene compounds, the vouacapane, since the compound is well described in the literature (Arriaga et al., 2000; Spindola et al., 2010).

The GC–MS analysis (Fig. 1B and C) together with literature data (Santos et al., 2010; Coelho et al., 2005; Arriaga et al., 2000; Adams, 1995) identifed eight sesquiterpene compounds (Table 1), three major sesquiterpene (E-cariofilene, γ-muurolene and biciclogermacrene), and nine vouacapane diterpenes (Table 1), in which four showed in major concentration (6α-acetoxyvouacapane, 6α,7β-dimetoxivouacapan-17-ene, 6α-acetoxy,7β-hidroxyvouacapane and 6α,7β-diacetoxycouacapane). The majority compounds were isolated by flash chromatograph and subjected to ^1H NMR, ^{13}C NMR and MS spectrometric analysis and comparing with literature data (Arriaga et al., 2000; Spindola et al., 2009; Coelho et al., 2005) which confirmed the structure of majority compounds sesquiterpenes **3** (E-cariofilene), **6** (γ-muurolene), **7** (biciclogermacrene) and vouacapane diterpenes **12** (6α-acetoxyvouacapane), **15** (6α,7β-dimetoxivouacapan-17-ene), **16** (6α-acetoxy-7β-hidroxyvouacapane), **17** (6α,7β-diacetoxycouacapane).

3.2. Acute OEPp administration reduces formalin-induced nociception

The results depicted in Fig. 2A and B show that OEPp (3–100 mg/kg) administered by intra-gastric (i.g.) gavage caused significant inhibition of both neurogenic and inflammatory phases of formalin-induced licking. The calculated mean ID_{50} value for the second phase was 9.88 (9.42–10.35) mg/kg and the inhibitions observed were 63 ± 5% for the first phase and 65 ± 7% for the second phase (100 mg/kg, i.g.). Furthermore, the OEPp (100 mg/kg, i.g.) also produced antiedematogenic effect with reduction of 52 ± 11% (Fig. 2C), when compared to the control group.

3.3. Acute and chronic OEPp administration reduces mechanical allodynia after hind paw incision

The results of Fig. 3A show that OEPp (0.1–100 mg/kg) administered by intra-gastric (i.g.) gavage caused a significant inhibition of plantar incision-induced allodynia when compared to the control group. The effect was maintained by 2 hours after

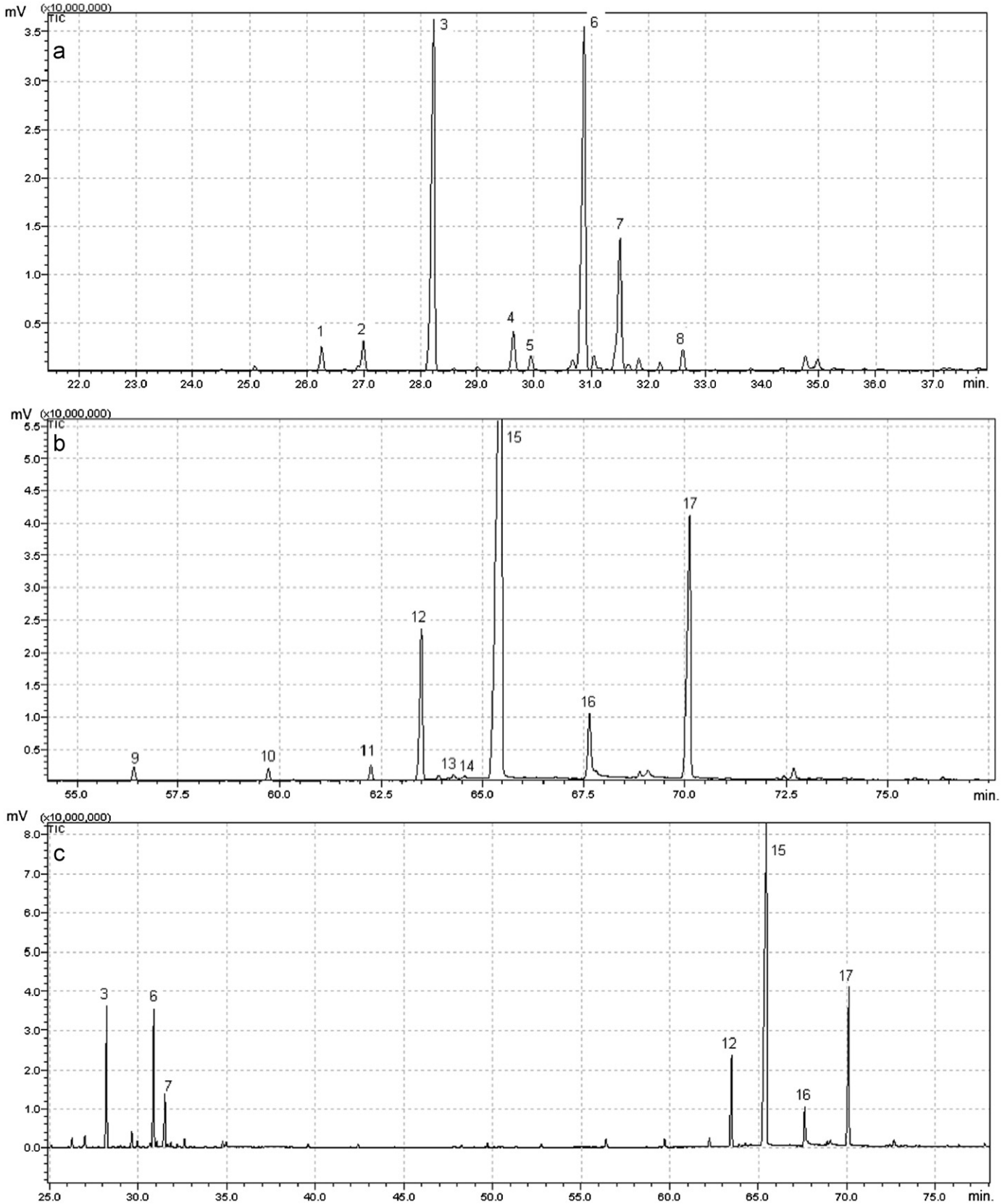

Fig. 1. Chromatograms of total ions the compounds from the extract of *P. pubescens* species in region of the sesquiterpene (A), diterpene (B) and full chromatogram (C).

treatment for the doses of 10 and 100 mg/kg, respectively. The observed inhibitions at the 2nd hour after treatment were 27 ± 1% and 35 ± 9% for the doses of 10 and 100 mg/kg, respectively. When administered chronically (once a day) for 6 day, OEPp (100 mg/kg, i.g.) significantly reduced the mechanical allodynia caused by plantar incision (inhibition from 47 ± 1% to 68 ± 1%, Fig. 3B).

Table 1
Data of mass chromatogram-fragment of compounds present in oil.

Compound	Fragment
1	105 (100), 204 (16), 189(5), 161 (95), 147 (5),133 (11), 119 (95), 91 (46), 81 (27), 69 (11), 55 (16), 41 (38)
2	93 (100), 189 (24), 175 (5), 161 (24), 147 (35), 133 (27), 121 (32), 105 (59), 81 (89), 79 (27), 67 (73), 53 (40), 41 (54)
3	41 (100), 204 (5), 189 (13), 175 (8), 161 (27), 147 (24), 135 (8), 133 (65), 119 (32), 105 (54), 93 (92), 79 (54), 69 (84), 55 (32), 53 (24).
4	93 (100), 204 (5), 161 (2,7), 133 (16), 121 (2,7), 107 (16), 105 (11), 80 (35), 79 (19), 67 (13), 65 (5), 55 (11), 53 (11), 41 (27)
5	41 (100), 204 (27), 189 (19), 176 (2,7), 161 (65), 147 (35), 133(54), 119 (49), 107 (57), 105 (76), 93 (57), 91 (95), 81 (51), 79 (62), 69 (51), 67 (54), 55 (40)
6	161 (100), 204 (16), 189 (2,7), 147 (5), 133 (19), 119 (35), 105 (68), 93 (27), 91 (54), 81 (38), 79 (35), 69 (13), 67 (16), 55 (19), 53 (11), 41 (38)
7	121 (100), 204 (13), 189 (11), 161 (27), 147 (8), 136 (19), 133 (8), 107 (51), 105 (38), 93 (81), 91 (43), 81 (27), 79 (40), 67 (27), 55 (19), 41 (46)
8	161 (100), 204 (43), 189 (19), 176 (2,7), 134 (51), 119 (57), 105 (57), 91 (38), 81 (24), 65 (8), 55 (13), 41 (27)
9	108 (100), 286 (40), 271 (11), 177 (11), 133 (8), 79 (13), 69 (11), 55 (13), 41 (19), 32 (8)
10	132 (100), 330 (3), 270 (38), 255 (13), 160 (11), 148 (24), 117 (43), 91 (30), 81 (27), 55 (30), 43 (59), 32 (27)
11	43 (100), 330 (19), 288 (5), 270 (19), 255 (57), 178 (46), 159 (27), 145 (24), 131 (35), 105 (81), 91 (57), 79 (54), 55 (51), 32 (32).
12	147 (100), 344 (5), 284 (13), 269 (5), 131 (13), 108 (11), 91 (5), 79 (8), 55 (8), 43 (16)
13	271 (100), 332 (3), 331 (16), 330 (78), 302 (46), 284 (11), 256 (8), 177 (49), 161 (13), 145 (27), 131 (73), 108 (89), 91 (59), 81 (59), 69 (73), 43 (59), 41 (89), 32 (73)
14	108 (100), 342 (24), 302 (30), 287 (59), 197 (27), 159 (19), 145 (40), 131 (27), 91 (19), 69 (40), 55 (35), 43 (59), 32 (59)
15	108 (100), 344 (2), 284 (11), 269 (22), 199 (11), 145 (22), 133 (24), 105 (22), 79 (19), 69 (19), 43 (40)
16	43 (100), 360 (32), 300 (65), 285 (22), 267 (13), 197 (27), 145 (92), 133 (65), 108 (89), 95 (32), 81 (40), 69 (49)
17	43 (100), 402 (11), 282 (89), 267 (13), 197 (84), 185 (13), 158 (30), 133 (54), 108 (30), 81 (16), 69 (22)

(**1**), α-copaene; (**2**), β-elemene; (**3**), E-cariofilene; (**4**), α-humulene; (**5**), alloaromandendrene; (**6**), γ-muurolene; (**7**), biciclogermacrene; (**8**), δ-cadinene; (**9**), vouacapane; (**10**), 6α-hidroxyvouacapane-7,17β-lactone; (**11**), 18α-hidroxyvouacapan-β7,17-lactone; (**12**), 6α-acetoxyvouacapane; (**13**), 6α-hidroxyvouacapan-β17- oic acid; (**14**), 6α-acetoxyvouacapan-17-ene; (**15**), 6α,7β-dimetoxivouacapan-17-ene; (**16**), 6α-acetoxy-7β-hidroxyvouacapane; (**17**), 6α,7β-diacetoxycouacapane.

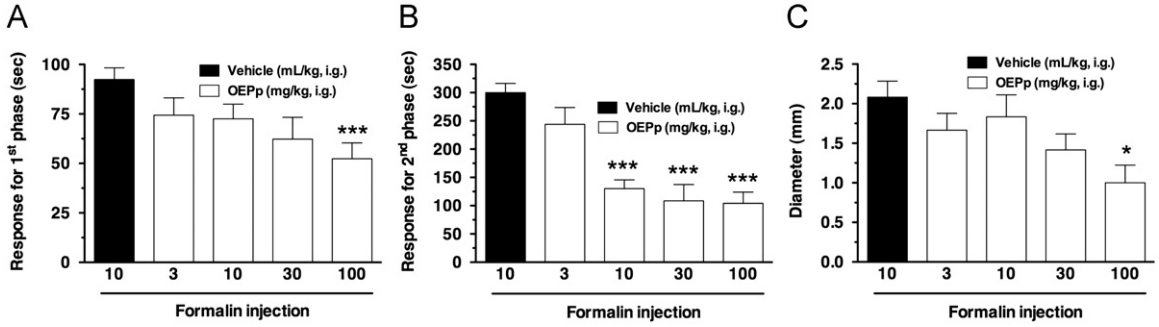

Fig. 2. Oleaginous extract *P. pubescens* administered intra-gastric reduces formalin-induced licking (first phase, panel A, and second phase, panel B) in mice and edema formation (diameter of paw, panel C). Each column represents the mean of the values obtained in 8 animals and the vertical lines indicate the S.E.M. The closed columns indicates the vehicle value (animals injected with vehicle) and the open columns correspond to animals treated with extract, the asterisks denote the significance levels, when compared with control group, (one-way ANOVA followed by Newman–Keuls' test) $^{*}P < 0.05$ and $^{***}P < 0.001$.

3.4. Acute and chronic OEPp administration reduces mechanical allodynia and thermal hyperalgesia after hind paw ischemia/reperfusion

3.4.1. Mechanical allodynia

The results presented in Fig. 4 show that OEPp (0.1–100 mg/kg) administered by i.g. gavage caused a significant inhibition of mechanical allodynia in CPIP mice, when compared to control group (Fig. 4A 2nd day and Fig. 4B 7th day). We observed that on the 2th or 7th days post-ischemia/reperfusion, OEPp produced antiallodynic effect that was maintained for 3 hours after treatment with the doses of 1, 10 and 100 mg/kg on the 2nd day after surgery; and at a dose of 100 mg/kg, on the 7th day after surgery (Fig. 4A 2nd day and Fig. 4B 7th day). The observed inhibitions at the 3rd hour after treatment, on the 2nd day, were 39 ± 1, 49 ± 1 and $45 \pm 1\%$ for the doses of 1, 10 and 100 mg/kg, respectively. Furthermore, on the 7th day, 3 h after treatment, the inhibition was $33 \pm 9\%$ for the dose of 100 mg/kg.

In the long-term treatment, OEPp (100 mg/kg, i.g.) was administered once a day for 5 day. The treatment significantly reduced mechanical allodynia (inhibition of $61 \pm 1\%$; Fig. 4C), and the assessments were always conducted 1 hour after treatment. Moreover, when the treatment was interrupted, the mice immediately exhibited a reestablishment of the mechanical allodynia. On the 14th day, the treatment was reinitiated and a significant inhibition of the mechanical allodynia was observed (inhibition of $51 \pm 1\%$, Fig. 4C).

3.4.2. Thermal hyperalgesia

The plantar incision surgery induced a decreased of the paw withdrawal latency during a thermal stimulus (heat and cold) in comparison to non-injured mice (Fig. 5A and B, respectively). Intra-gastric pretreatment with OEPp (100 mg/kg) reduced the thermal hyperalgesia induced by the incision. Furthermore, the latency to paw withdrawal was increased by 28 and 64%, for the heat and cold assessments, respectively.

3.4.3. Locomotor activity

OEPp treatment (1–100 mg/kg, i.g., given 60 min beforehand), did not alter mouse ambulation in the open field test. The crossing numbers after administration were 94.0 ± 3.6, 91.5 ± 8.7, 94.8 ± 9.6 and 96.4 ± 11.4 for the control group and the groups receiving 1, 10 or 100 mg/kg of OEPp, respectively (data not shown).

4. Discussion

Sucupira seeds are commercially available in the Brazilian medicinal plant market. The crude alcoholic extracts of this plant

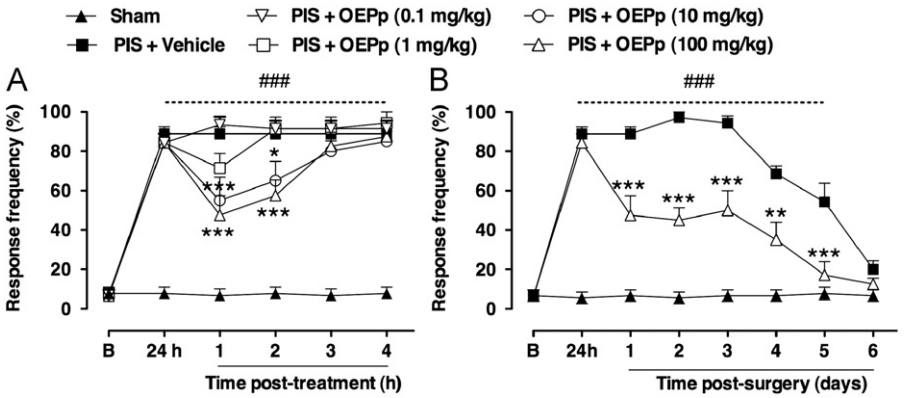

Fig. 3. Oleaginous extract *P. pubescens* administered orally decreases postoperative pain. Time-course of the antiallodynic effect of OEPp on the mechanical nociception (panel A). Chronic administration with OEPp on the mechanical allodynia (panel B). Each point represents the mean of the values obtained in 8 animals and the vertical lines indicate the S.E.M. The closed points indicates the vehicle value (animals injected with vehicle) and the open points correspond to animals treated with extract, the asterisks denote the significance levels, when compared with control group or $^{###}P < 0.001$ when compared with sham-operated group (Two-way ANOVA followed by Bonferroni's test) $^{*}P < 0.05$ and $^{***}P < 0.001$. B: baseline withdrawal threshold; PIS: Plantar incision surgery.

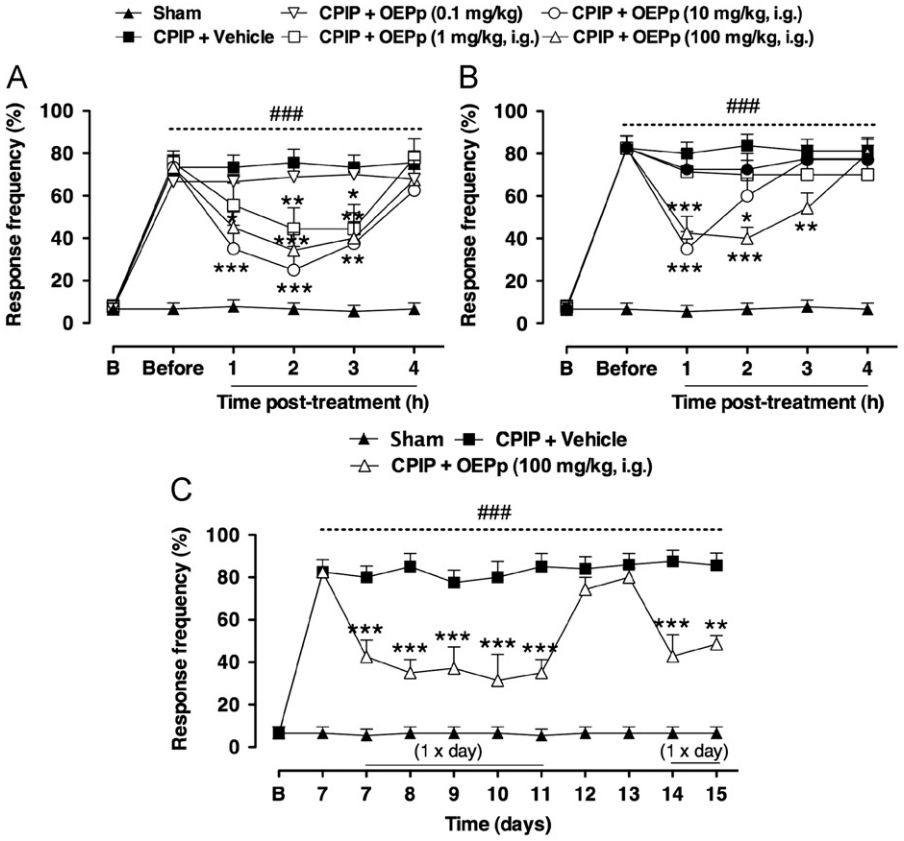

Fig. 4. Oleaginous extract *P. pubescens* administered intra-gastric reduces pain in complex regional pain syndrome type-I animal model. Time-course of the antinociceptive effect of OEPp (0.1–100 mg/kg., i.g.), on the mechanical allodynia (2nd day, panel A; 7th day, panel B). Chronic administration wit OEPp (100 mg/kg., i.g.) on the mechanical allodynia (panel C). Each point represents the mean of the values obtained in 8 animals and the vertical lines indicate the S.E.M. The closed points indicates the vehicle value (animals injected with vehicle) and the open points correspond to animals treated with extract, the asterisks denote the significance levels, when compared with control group or $^{###}P < 0.001$ when compared with sham-operated group (Two-way ANOVA followed by Bonferroni's test) $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. B: baseline withdrawal threshold; CPIP: Chronic post-ischemic pain.

are used in folk medicine as anti-inflammatory, analgesic and anti-rheumatic preparations (Lorenzi, 1998; Pio Correa, 1975). The present study confirms and extends previous data from the literature and demonstrates that systemic (oral) administration of oleaginous extract of *P. pubescens* (OEPp) elicits a potent and dose-dependent inhibition of the nociceptive behavioral response in animal models of acute and chronic pain. The most relevant results in the work are that (1) intra-gastric administration of OEPp caused significant inhibition against both phases of the pain response to the intraplantar injection of formalin and edema formation; (2) acute and chronic intra-gastric administration of OEPp also reduced mechanical allodynia after incision or ischemia/reperfusion of hind paw, to our knowledge this is the first report of its kind in the literature; (3) OEPp increased the response latency in the hot- and cold-plate tests after paw incision and (4) the dose of OEPp that caused significant

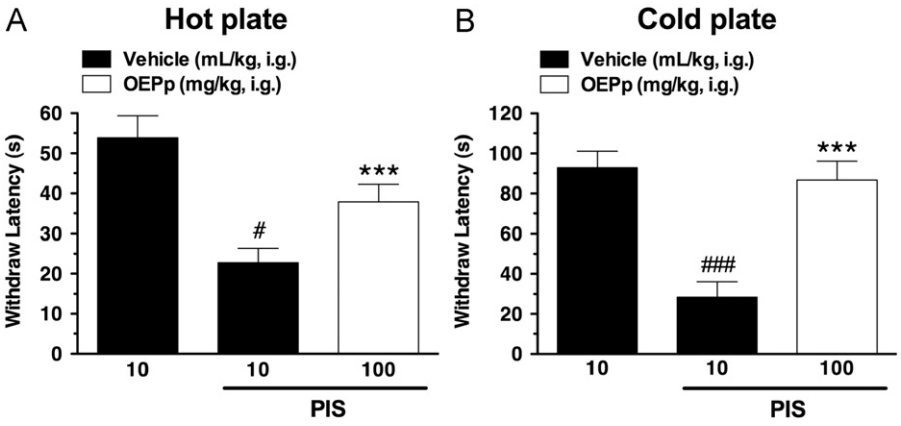

Fig. 5. Oleaginous extract *P. pubescens* decreases thermal hyperalgesia in postoperative pain animal model. Heat and cold hyperalgesia were evaluated 60 min after treatment with OEPp (panel A and B, respectively) 24 h after incision of hind paw. Each point represents the mean of the values obtained in 8 animals and the vertical lines indicate the S.E.M. The closed columns indicates the vehicle value (animals injected with vehicle) and the open columns correspond to animals treated with OEPp (100 mg/kg., i.g.), the asterisks denote the significance levels, when compared with control group or $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ when compared with sham-operated group (one-way ANOVA followed by Newman–Keuls' test) $^{***}P < 0.001$. PIS: Plantar incision surgery.

antiallodynic effect did not produce any statistically significant motor dysfunction or any detectable side-effect.

P. pubescens, popularly known in Brazil as 'sucupira branca', has been widely used by domestic medicine as an anti-inflammatory (Coelho et al., 2005). Data in the literature has demonstrated that the extract of *P. emarginatus* inhibits both neurogenic and inflammatory phases observed in the formalin test (Galceran et al., 2011). Corroborating these data, we also demonstrated that OEPp caused significant and dose-related antinociception when administered orally against both neurogenic (early phase) and inflammatory (late phase) pain responses caused by formalin injection. Furthermore, OEPp also attenuated partially but significantly the edema formation associated with the late phase of the formalin response. It has been demonstrated that kinins, prostanoids and serotonin, but not tachykinin, play an important modulatory role in controlling formalin induced edema formation (Corrêa et al., 1996; Santos and Calixto, 1997).

Phytochemical studies of the *Pterodon* genus have shown the presence of alkaloids, isoflavones and diterpenes. In this regard, it has been demonstrated that furanditerpenes identified from *Pterodon* fruits (Mahjan and Monteiro, 1973; Spindola et al., 2009) contribute to the anti-inflammatory and antinociceptive properties of *P. pubescens* seed oil (Nunan et al., 1982; Coelho et al., 2005). Furthermore, diterpenes 6α-hydroxyvouacapan-7β-17β-lactone and 6α,7β-dihydroxyvouacapan-17β-oate methyl ester found in *P. emarginatus* and *P. polygalaeflorus* seeds also appears to contribute with the anti-inflammatory activity of these species (Nunan et al., 1982).

Another interesting data of the present study is the demonstration, for the first time, that acute or prolonged treatment of mice with OEPp was able to reduce, in a great manner, mechanical allodynia in a mouse postoperative pain model. Moreover, the antiallodynic effect of OEPp lasted for up to 2 h when analyzed in the mechanical allodynia caused by paw incision. Postoperative pain in humans can be mimicked by paw incision in rats (Brennan et al., 1996). Thus, the plantar incision model may also be useful for predicting postoperative analgesic effects of investigational agents.

Several approaches have been used to reduce postoperative pain, including administration of systemic opioids and non-steroidal anti-inflammatory drugs, epidural or spinal infusion of local anesthetics, and peripheral nerve block. It has been suggested that postoperative pain produces a unique pharmacology of analgesia compared with other sustained pain models. For example, it was reported that reuptake inhibition of NA and 5-HT (milnacipran) in the spinal cord inhibits postoperative pain (Obata et al., 2001). In agreement with these results, previous studies using the acetic acid test have shown that 6α,7β-dihydroxyvouacapan-17β-oate methyl ester (crude alcoholic extracts obtained from *P. pubescens* Benth) presented a significant loss of activity after p-chlorophenylalanine methyl ester hydrochloride (PCPA, 5-HT synthesis inhibitor) treatment, suggesting that the mechanisms of action could be related to either the synthesis or release of serotonin (Spindola et al., 2011).

Neurotransmitters released by noxious stimuli may contribute to the enhanced excitability following surgical injury. Of late, particular attention has been paid to the action of excitatory amino acids (EAAs) (Coderre and Melzack, 1992). Several findings were derived from experiments conducted using the post-operative model of pain and spinal EAAs receptor antagonists (Zahn et al., 1998; Zahn and Brennan, 1998). The intrathecal non-NMDA receptor antagonist returned the withdrawal threshold nearly to pre-incision levels. Furthermore, the activation of spinal non-NMDA, AMPA-kainate receptors were found to mediate guarding behavior and the reduced withdrawal threshold that develops after an incision (Zahn et al., 1998). In surgery, after tissue injury and inflammation, nociceptors are sensitized in such a way that a slight stimulation becomes painful. In postoperative pain, the sensitization of Aδ and C fibers occurs at the same mechanical stimuli intensity. It has been suggested that TRPV1 is important for generation of thermal hyperalgesia after incision (Pogatzki-Zahn et al., 2005). In agreement with these reports, it has been demonstrated that geranylgeraniol and 6α,7β-dihydroxyvouacapan-17β-oate methyl ester isolated from *P. pubescens* Benth produces antinociceptive activity when evaluated in the capsaicin and glutamate animal experimental models (Spindola et al., 2010).

Complex Regional Pain Syndrome Type I (CRPS I) is a severe, disabling, and painful disease which may occur in an extremity after a trauma or injury. Clinical features include spontaneous and stimulus-evoked pain, edema, vasomotor and sudomotor disturbances, motor dysfunction, and trophic changes, without evidence of peripheral nerve injury (Mersky and Bogduk, 1994; Stanton-Hicks et al., 1995). The experimental model of CRPS-I produced by IR of paw in rat or mice are able to mimic important symptoms observed in patients with peripheral neuropathy and are employed in behavioral research (Millecamps et al., 2010; Coderre et al., 2004). Furthermore, it has been demonstrated that only high doses of morphine, dexamethasone and pregabalin partially reduced mechanical allodynia 2 day post-ischemia/

reperfusion, while other treatments (ibuprofen, acetaminophen, amitriptyline) were not effective. In seven days post-ischemia/reperfusion only the highest dose of pregabalin reduced mechanical allodynia (Millecamps and Coderre, 2008).

Also of interest are the results showing that acute or prolonged systemic (oral) treatments of animals with OEPp are effective in preventing the mechanical allodynia induced by ischemia/reperfusion (IR) of the hind paw. Responses of rats or mice with chronic post-ischemia pain to classical pharmacological treatments exhibit a profile very similar to the profile in human complex regional pain syndrome-I patients, with an early period sensitive to anti-inflammatory drugs and morphine, and a later period similar to neuropathic pain patients, in which a resistance develops to these treatments. To assess the clinical potential of OEPp as an anti-inflammatory and -neuropathic drug, we treated mice with OEPp on the 2nd and 7th days after IR. OEPp was effective on both days, which similar to the effectiveness of others drugs, i.e. pregabalin, with effects in various pain conditions. Moreover, the anti-allodynic action of OEPp lasted for up to 3 h.

Interestingly, the anti-allodynic response caused by OEPp produced not accumulative effect. This conclusion derives from data showing that (1) the withdrawal of OEPp was followed by complete return to baseline mechanical allodynia, and (2) a new intra-gastric treatment with OEPp, once a day, produced very similar and pronounced anti-allodynic effects. To our knowledge this is the first report of its kind in the literature.

5. Conclusions

In summary, the results of the present study demonstrate for the first time that OEPp reduced mechanical allodynia in post-operative pain and complex regional pain syndrome type-I animal models and increased the latency response in hot- and cold-plate tests after paw incision (postoperative pain). In addition, the present work confirms and extends previous data from the literature showing that systemic administration of OEPp caused significant inhibition against both phases of the pain response to the intraplantar injection of formalin and edema formation. This could provide a rationale for the use of this plant to treat pain disorders in folk medicine.

Conflict of interest

The authors declare that there are no conflicts of interest.

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